REMARKS

Applicants acknowledge, with appreciation, that the Examiner has rejoined groups VI and I, and that the following are under consideration as the elected species: 1,2-dimethyl-9,10-benzanthracene, 7,12-dimethylbenz[a]anthracene (DMBA), and 1,2-dimethylanthracene. However, Applicants note that 7,12-dimethylbenz[a]anthracene (DMBA) is not among the specific species recited in the claims for which an election was required. It appears that the Examiner is inferring this compound from claim 3, for example. However, DMBA is not a species that is defined by originally filed claim 3. Applicants herein add new claims 72-82 and cancel claims 2 and 3. Thus, the claims under consideration are claims 1, 4-13, 22-24, 27-29, 68, 70, and 72-82.

Claim Amendments

Claim 1 has been amended to incorporate the features of claims 2 and 3. Therefore, Applicants respectfully request cancellation of claims 2 and 3 without prejudice. Claims 4-13 have been amended to depend from claim 1, rather than claim 3, which is cancelled herein.

Claim 10 has been amended to be drawn to compounds in which the R_1, R_2, R_9, and R_{10} groups are substituted, as in the claimed compound, 1,2-dimethyl-9,10-benzanthracene.

Claim 12 has been amended to be drawn to compounds in which the R_3, R_4, R_9 and R_{10} groups are substituted, as in the claimed compound, 9-hydroxymethyl-10-methylanthracene-3,4-diol.

Claim 70 has been amended to remove dependency from Claim 69, which has been withdrawn from consideration.

No new matter is introduced by any of the forgoing amendments.

35 U.S.C. §112, First Paragraph (Written Description)

The Office Action rejects claims 1-13, 22-24, 27-29, 68, and 70 as allegedly lacking written description. However, the Examiner indicated that there was sufficient written description for a "genotoxic mutation in a mismatch repair gene in a cell *in vitro* , which is produced by exposing the cell to anthracene *in vitro* and employoing an MMR-sensitive report gene assay or a polynucleotide repeat marker nucleic acid binding assay to determine the presence of the mutation in said gene." The Office Action also states that there is sufficient written description for "genotoxic mutation in a mismatch repair gene in a non-human cell *in vivo* comprising administering to the cell an effective amount of anthracene, and employing an MMR-sensitive report gene assay to determine the presence of the mutation in said gene." These statements mischaracterize the invention somewhat. To be clear, the anthracene inhibits the activity of mismatch repair, but it is believed that unlike other genotoxic compounds, it does not itself induce mutations. Rather, the cell accumulates genome-wide mutations due to the fact that during DNA replication, the cell cannot repair the mismatches that occur. Thus, the invention comprises methods of mutating genes of cells *in vitro* and screening for genetic mutations using the genetic assays, such as those described.

Therefore, Applicants submit that the amended claims and newly added claims are supported by an adequate written description, as these claims have been provided following the guidelines proposed by the Examiner. These claims include:

(a) methods of making the cell hypermutable (*i.e.*, unable to repair mismatches in the DNA) by administering to the cells *in vitro* an anthracene as defined by the claims;

(b) methods of generating a mutation in a gene of interest by administering an anthracene as defined by the claims, allowing mutations to accumulate and selecting cells comprising a mutation in the gene of interest;

(c) methods of making non-human cells hypermutable (*i.e.*, unable to repair mismatches in the DNA) *in vivo* by administering to the cells an anthracene as defined by the claims;

(d) methods of generating a mutation in a gene of interest *in vivo* by administering an anthracene as defined by the claims, allowing mutations to accumulate and selecting cells comprising a mutation in the gene of interest (which may cover non-human applications).

The invention provides a general methods which are applicable to all species having mismatch repair genes. As described in the Specification at page 2, lines 3-8, MMR is a conserved process in a diverse array of species including bacteria, yeast, *Drosophila*, and mammals (including mice and humans). Thus, one of skill in the art would know the broad applicability of the methods of the invention, and the teachings of the Specification provide support for the use of the methods in a broad array of species.

Furthermore, one of skill in the art is well acquainted with methods of genetic analysis to determine whether a gene of interest comprises a mutation. The assertion in

the Office Action that there is not an adequate written description of the assays to determine alterations of a gene of interest is against the weight of evidence in the prior art which is replete with methods for analyzing genes in cells. The Applicants provide a novel method of inducing genome wide mutations in cells, which had been problematic using traditional mutagens.

The Examiner also argues that beneficial effects in genes of interest "appears to contradict the toxic effects of anthracene" aside from being an unsupported presumption on the part of the Examiner, ignores the fact that beneficial mutations may be selected. In fact, selection of beneficial traits is generally the goal of the skilled artisan practicing the methods of the invention, and such is immediately apparent to one of skill in the art.

Finally, the Applicants have demonstrated the utility of the invention with actual reduction to practice and guidelines to use the method in other applications. To require a description of each and every gene that may be mutated and analyzed is unnecessary to satisfy the written description requirement as one of skill in the art can easily understand the broad utility of the methods and would be able to apply the methods for analyzing a gene of interest to him.

Applicants earnestly submit that adequate written description for the claims, as amended, is provided by the Specification. Thus, withdrawal of the rejection under 35 U.S.C. §112, first paragraph is solicited.

35 U.S.C. §112, First Paragraph (Enablement)

The Office Action rejects claims 1-13, 22-24, 27-29, 68 and 70. Without conceding the correctness of the Examiner's rejection, Applicants have amended the claims along the guidelines proposed by the Examiner as to what is enabled by the Specification (as set forth in the Office Action at page 7), with the correction to the characterization of the invention as set forth above.

35 U.S.C. §102(b)/103(a)

Turning to the merits of the invention, the Office Action rejects the claims under 35 U.S.C. §102(b)/103(a), applying a few references. The combinations will be addressed in turn.

1. Chakravarti and Nakazawa

The Office Action has rejected claims 1-13, 22-24, 27-29, 68 and 70 as anticipated, or, in the alternative, obvious over Chakravarti *et al.* (1995) *Proc. Natl. Acad. Sci. USA* 92:10422-10426 ("Chakravarti") or Nakazawa *et al.* (1990) *Mol. Carcinogenesis* 3:202-209 ("Nakazawa"). Both Chakravarti and Nakazawa teach the use of 7,12-dimethylbenz[a]anthracene as the tumor-inducing agent. Chakravarti notes at page 10423 that the structural formula for 7,12-dimethylbenz[a]anthracene is a compound with a core of four rings (which the Examiner likely considers an anthracene in which the 7 and 12 positions are fused in a polycyclic ring). However, the specification and claims are drawn to anthracenes in which positions 1-10 are individually substituted. Thus, the 7,12 polycyclic ring is outside the scope of the claims.

Moreover, the chemical inhibitors of mismatch repair taught in the present application are distinct from DMBA. The chemical inhibitors taught in the present application inhibit mismatch repair such that subsequent exposure to traditional mutagens, such as DMBA, will not result in the increased rate of mutagenesis seen in mismatch repair proficient cells (see Specification at page 22, lines 9-29). Thus, neither Chakravarti nor Nakazawa anticipate the claims, nor render them obvious.

2. Zhang in view of Hoorn

The Office Action also rejects claims 1-13, 22-24, 27-29, 68 and 70 under 35 U.S.C. 103(a) over U.S. Patent Publication No. US2002/0064879 A1 to Zhang ("Zhang") in view of Hoorn *et al.* (1993) 8(1):7-10 ("Hoorn"). The Office Action alleges that one would be motivated to combine the method taught in Zhang using the mutagen taught by Hoorn. As noted above, Hoorn teaches the use of DMBA, which is outside the scope of the claims and further is a fundamentally different type of compound than the mismatch repair inhibitors of the invention. Thus, the hypothetical combination of Zhang and Hoorn fail to achieve the invention as instantly claimed.

3. Chakravarti or Nakazawa in view of Kotiloglu

The Office Action also rejects claims 1-13, 22-24, 27-29, 68 and 70 under 35 U.S.C. 103(a) over Chakravarti or Nakazawa in view of Kotiloglu and Oner (1993) *Doga – Turkish J. Med. Sci.* 18/2:115-126 ("Kotiloglu"). Kotiloglu used 1,2-dimethyl-9,10-benzanthracene to induce tumors in mice. However, like DMBA, 1,2-dimethyl-9,10-benzanthracene contains a polycyclic group rather than have independent substitutions.

Again, 1,2-dimethyl-9,10-benzanthracene acts as a mutagen rather than as a mismatch repair inhibitor and, as such, is distinct from the chemical inhibitors of the invention. Thus, even in the hypothetical combination of Chakravarti or Nakazawa and Kotiloglu, the collective teachings fail to achieve the instantly claimed invention. Moreover, as the compounds of the invention and the cited compounds function in a different manner, it is clear that mere substitution of groups on the cited compounds would achieve the characteristics of the compounds in the claimed methods. Thus, Applicants respectfully submit that the hypothetical combination of references does not render the claims obvious.

Applicants submit that the claims are in condition for allowance and are neither anticipated nor obvious in view of the cited art. Applicants respectfully request allowance of the claims as amended.

Respectfully submitted,

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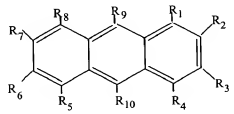
Version with Markings to Show Changes Made

In the Claims

1. (Amended) A method for making a hypermutable cell *in vitro* comprising exposing a cell to an inhibitor of mismatch repair, wherein said inhibitor is an anthracene, [an ATPase inhibitor, a nuclease inhibitor, a polymerase inhibitor, or an antisense oligonucleotide that specifically hybridizes to a nucleotide encoding a mismatch repair protein] wherein said anthracene has the formula:



wherein R_1-R_{10} are independently hydrogen, hydroxyl, amino, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, O-alkyl, S-alkyl, N-alkyl, O-alkenyl, S-alkenyl, N-alkenyl,O-alkynyl, S-alkynyl, N-alkynyl, aryl, substituted aryl, aryloxy, substituted aryloxy, heteroaryl, substituted heteroaryl, aralkyloxy, arylalkyl, alkylaryl, alkylaryloxy, arylsulfonyl, alkylsulfonyl, alkoxycarbonyl, aryloxycarbonyl, guanidino, carboxy, an alcohol, an amino acid, sulfonate, alkyl sulfonate, CN, NO_2, an aldehyde group, an ester, an ether, a crown ether, a ketone, an organosulfur compound, an organometallic group, a carboxylic acid, an organosilicon or a carbohydrate that optionally contains one or more alkylated hydroxyl groups;

wherein said heteroalkyl, heteroaryl, and substituted heteroaryl contain at least one heteroatom that is oxygen, sulfur, a metal atom, phosphorus, silicon or nitrogen; and

wherein said substituents of said substituted alkyl, substituted alkenyl, substituted alkynyl, substituted aryl, and substituted heteroaryl are halogen, CN, NO_2, lower alkyl,

aryl, heteroaryl, aralkyl, aralkyloxy, guanidino, alkoxycarbonyl, alkoxy, hydroxy, carboxy and amino;
and wherein said amino groups optionally substituted with an acyl group, or 1 to 3 aryl or lower alkyl groups.

Please cancel claims 2 and 3 without prejudice.

4. (Amended) The method of claim [3] 1 wherein R_5 and R_6 are hydrogen.

5. (Amended) The method of claim [3] 1 wherein R_1-R_{10} are independently hydrogen, hydroxyl, alkyl, aryl, arylaklyl, or hydroxyalkyl.

6. (Amended) The method of claim [3] 1 wherein R_1-R_{10} are independently hydrogen, hydroxyl, methyl, ethyl, propyl, isopropyl, butyl, isobutyl, phenyl, tolyl, hydroxymethyl, hydroxypropyl, or hydroxybutyl.

7. (Amended) The method of claim [3] 1 wherein said anthracene is selected from the group consisting of 1,2-dimethylanthracene, 9,10-dimethyl anthracene, 7,8-dimethylanthracene, 9,10-diphenylanthracene, 9,10-dihydroxymethylanthracene, 9-hydroxymethyl-10-methylanthracene, dimethylanthracene-1,2-diol, 9-hydroxymethyl-10-methylanthracene-1,2-diol, 9-hydroxymethyl-10-methylanthracene-3,4-diol, and 9, 10-di-m-tolyanthracene.

8. (Amended) The method of claim [3] 1 wherein R_3, R_4, R_5, R_6, R_7, R_8, R_9 and R_{10} are hydrogen.

9. (Amended) The method of claim [3] 1 wherein R_1, R_2, R_3, R_4, R_5, R_6, R_7 and R_8 are hydrogen.

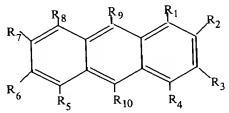
10. (Amended) The method of claim [3] 1 wherein [R_1, R_2,] R_3, R_4, R_5, R_6, R_7 and R_8 are hydrogen.

11. (Amended) The method of claim [3] 1 wherein R_1, R_2, R_3, R_4, R_5, R_6, R_9 and R_{10} are hydrogen.

12. (Amended) The method of claim [3] 1 wherein R_1, R_2, [R_3, R_4,] R_5, R_6, R_7 and R_8 are hydrogen.

13. (Amended) The method of claim [3] 1 wherein R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_8 and R_{10} are hydrogen.

23. (Amended) A method for generating a mutation in a gene of interest comprising exposing a cell comprising said gene of interest to a chemical mismatch repair inhibitor *in vitro*, wherein said mismatch repair inhibitor is an anthracene having the formula:



wherein R_1-R_{10} are independently hydrogen, hydroxyl, amino, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, O-alkyl, S-alkyl, N-alkyl, O-alkenyl, S-alkenyl, N-alkenyl,O-alkynyl, S-alkynyl, N-alkynyl, aryl, substituted aryl, aryloxy, substituted aryloxy, heteroaryl, substituted heteroaryl, aralkyloxy, arylalkyl, alkylaryl, alkylaryloxy, arylsulfonyl, alkylsulfonyl, alkoxycarbonyl, aryloxycarbonyl, guanidino, carboxy, an alcohol, an amino acid, sulfonate, alkyl sulfonate, CN, NO_2, an aldehyde group, an ester, an ether, a crown ether, a ketone, an organosulfur compound, an organometallic group, a carboxylic acid, an organosilicon or a carbohydrate that optionally contains one or more alkylated hydroxyl groups;

wherein said heteroalkyl, heteroaryl, and substituted heteroaryl contain at least one heteroatom that is oxygen, sulfur, a metal atom, phosphorus, silicon or nitrogen; and

wherein said substituents of said substituted alkyl, substituted alkenyl, substituted alkynyl, substituted aryl, and substituted heteroaryl are halogen, CN, NO_2, lower alkyl, aryl, heteroaryl, aralkyl, aralkyloxy, guanidino, alkoxycarbonyl, alkoxy, hydroxy, carboxy and amino;

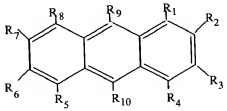
and wherein said amino groups optionally substituted with an acyl group, or 1 to 3 aryl or lower alkyl groups; and

testing said cell to determine whether said gene of interest comprises a mutation.

70. (Amended) The method of claim 68 [or 69] wherein said mutagen is selected from the group consisting of N-methyl-N'-nitro-N-nitrosoguanidine, methane sulfonate, dimethyl sulfonate, O-6-methyl benzadine, ethyl methanesulfonate, methylnitrosourea, and ethylnitrosourea.

Please add the following new claims:

72. A method for making a hypermutable non-human organism comprising exposing at least one cell of said organism to an inhibitor of mismatch repair, wherein said inhibitor is an anthracene, wherein said anthracene has the formula:



wherein R_1-R_{10} are independently hydrogen, hydroxyl, amino, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, O-alkyl, S-alkyl, N-alkyl, O-alkenyl, S-alkenyl, N-alkenyl,O-alkynyl, S-alkynyl, N-alkynyl, aryl, substituted aryl, aryloxy, substituted aryloxy, heteroaryl, substituted heteroaryl, aralkyloxy, arylalkyl, alkylaryl, alkylaryloxy, arylsulfonyl, alkylsulfonyl, alkoxycarbonyl, aryloxycarbonyl,

guanidino, carboxy, an alcohol, an amino acid, sulfonate, alkyl sulfonate, CN, NO_2, an aldehyde group, an ester, an ether, a crown ether, a ketone, an organosulfur compound, an organometallic group, a carboxylic acid, an organosilicon or a carbohydrate that optionally contains one or more alkylated hydroxyl groups;

wherein said heteroalkyl, heteroaryl, and substituted heteroaryl contain at least one heteroatom that is oxygen, sulfur, a metal atom, phosphorus, silicon or nitrogen; and

wherein said substituents of said substituted alkyl, substituted alkenyl, substituted alkynyl, substituted aryl, and substituted heteroaryl are halogen, CN, NO_2, lower alkyl, aryl, heteroaryl, aralkyl, aralkyloxy, guanidino, alkoxycarbonyl, alkoxy, hydroxy, carboxy and amino; and

wherein said amino groups optionally substituted with an acyl group, or 1 to 3 aryl or lower alkyl groups.

73. The method of claim 72 wherein R_5 and R_6 are hydrogen.

74. The method of claim 72 wherein R_1-R_{10} are independently hydrogen, hydroxyl, alkyl, aryl, arylaklyl, or hydroxyalkyl.

75. The method of claim 72 wherein R_1-R_{10} are independently hydrogen, hydroxyl, methyl, ethyl, propyl, isopropyl, butyl, isobutyl, phenyl, tolyl, hydroxymethyl, hydroxypropyl, or hydroxybutyl.

76. The method of claim 72 wherein said anthracene is selected from the group consisting of 1,2-dimethylanthracene, 9,10-dimethyl anthracene, 7,8-dimethylanthracene, 9,10-diphenylanthracene, 9,10-dihydroxymethylanthracene, 9-hydroxymethyl-10-methylanthracene, dimethylanthracene-1,2-diol, 9-hydroxymethyl-10-methylanthracene-1,2-diol, 9-hydroxymethyl-10-methylanthracene-3,4-diol, and 9, 10-di-m-tolyanthracene.

77. The method of claim 72 wherein R_3, R_4, R_5, R_6, R_7, R_8, R_9 and R_{10} are hydrogen.

78. The method of claim 72 wherein R_1, R_2, R_3, R_4, R_5, R_6, R_7 and R_8 are hydrogen.

79. The method of claim 72 wherein R_3, R_4, R_5, R_6, R_7 and R_8 are hydrogen.

80. The method of claim 72 wherein R_1, R_2, R_3, R_4, R_5, R_6, R_9 and R_{10} are hydrogen.

81. The method of claim 72 wherein R_1, R_2, R_5, R_6, R_7 and R_8 are hydrogen.

82. The method of claim 72 wherein R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_8 and R_{10} are hydrogen.